IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re app. of:	**Florence Henry et al.**	Examiner:	**Catheryne Chen**
App. No.:	**10/578,344** Conf. No.: 6599	Group Art Unit:	**1655**
Filed:	**May 5, 2006**	Docket No.:	**C 2892 PCT/US (P40090 USA)**

For: **COMPOSITION CONTAINING AN EXTRACT OF THE FRUIT OF SCHISANDRA CHINENSIS AND PROCESS FOR PRODUCING SAME**

DECLARATION OF MARIE-FRANCE ZAMBAUX UNDER 37 C.F.R. 1.132

I, Marie-France Zambaux, declare and state that:

1. I am currently employed by Cognis GmbH as a R&D Operations Manager. I have held this position for about 4 years. I hold a B.S. degree (1994) in Biochemistry from University of Nancy I, Raymond Poincaré, France, and a Ph.D. degree (1998) in Biological and Medical Engineering from University of Nancy I, Raymond Poincaré, France. I have extensive experience with natural products, natural product extracts, and cosmetic and pharmaceutical compositions comprising natural product extracts.

2. I am familiar with the prosecution history of U.S. Patent Application Serial No. 10/578,344 and have reviewed the claims currently pending therein. I understand that the claims of this application have been rejected as allegedly being anticipated and/or obvious in view of Kim et al. (J. Chromatographic Science, 1999, 37, 457-461), Ikeda et al. (JP 06279256), Newmark et al. (US 6,242,012), and Sung et al. (WO 2001/041778). I have reviewed these publications and the compositions disclosed therein.

3. The Examiner has stated in the Final Office Action dated September 15, 2009, that

> "Applicant argues that there is unexpected superior performance from supercritical extraction. In response to Applicant's argument, there is no data to support the allegation. Applicant need [sic] to show that supercritical extract of

Schisandra chinensis is better than other types of Schisandra chinensis extract. Thus, unexpected result is unpersuasive" (Final Office Action, page 7, middle).

4. In order to demonstrate the unexpected superior performance of the supercritical solvent extract, I have conducted or supervised the performance of tests as described herein.

5. Extracts of Schisandra chinensis were prepared as follows. The supercritical CO_2 extract of Schisandra chinensis (**SC-extract**) was prepared according to the example described on page 22 of WO 2005/044289, which is equivalent to the procedure found on page 26 of the present specification as originally filed.

The aqueous extract Schisandra chinensis (**AQ-extract**) was prepared according to the following procedure:

1 kg of dried and powered Schisandra chinensis berries were introduced into a beaker containing 1 L of distilled water. The mixture was then stirred at 85°C for one hour. The solids were removed by centrifugation (4200 rpm for 15 minutes) and filtration. The solution was again filtered to obtain a crude solution of the extract (dried extract of the liquid: 2.6%). The water was removed by spray drying, and the yield obtained was 3% based on the weight of the dried berries.

6. The anti-aging efficacy was evaluated *in vitro* in a cell culture of human fibroblasts. The test is identical to that of Example 3, page 25 of WO 2005/044289, which is equivalent to the procedure on pages 29-30 of the present specification as originally filed.

The assays were carried out in triplicate and repeated three to four times. The results are calculated in reference to a standard range for proteins, ATP and DNA, and are presented in % control (standard medium in the absence of added extract). The results are expressed as a mean with standard deviation (SD), and were statistically evaluated by the student t test.

7. The *in vitro* test system was validated using Foetal Calf Serum (FCS, Table 1), which provided statistically significant stimulation of all three parameters at 1% and 3% FCS. Results are given in % control (mean +/- SD on 1 assay in triplicate).

Table 1

Dose of Foetal Calf Serum (=FCS) :		**Mean**	SD	**p of Student t test**
Proteins	**0%**	**100**	3.1	
	1%	**142.3**	3.6	0.00010
	3%	**182.9**	20.9	0.00243
ATP	**0%**	**100**	2.9	
	1%	**155**	8.5	0.00045
	3%	**201.2**	11.4	0.00012
DNA	**0%**	**100**	5.2	
	1%	**119.8**	4.4	0.00744
	3%	**135.7**	3.0	0.00051

Statistics: student t test referring to the control: $p < 0.05$ = significant effect; $p < 0.01$ = very significant effect.

8. In Summary, Table 1 clearly demonstrates that the reference substance, Foetal Calf Serum at 1% and 3% significantly enhances the levels of cellular proteins, ATP and DNA. Therefore, the *in vitro* test system is validated.

9. For comparison of the Schisandra chinensis extracts in the *in vitro* human fibroblast cell culture assay, each extract was solubilized as a 1% stock solution in DMSO (dimethylsulfoxide) and then diluted directly into standard cell culture medium at the concentrations indicated in Table 2. As a control, the solvent DMSO was tested alone at the corresponding concentrations.

Table 2

Dose of tested extract		AQ-extract			SC-extract		
		Mean	SD	**p of student t test**	**Mean**	SD	**p of student t test**
Proteins	**0%**	**100**	3.1	-	**100**	3.1	-
	0.0003%	**93.9**	7.9	0.27635	**121.9**	8.1	0.01167
	0.001%	**92.4**	7.0	0.15948	**151.7**	7.9	0.00046
	0.003%	**92.7**	5.3	0.10688	**155.1**	4.0	0.00004
ATP	**0%**	**100**	2.9	-	**100**	2.9	-
	0.0003%	**91.3**	2.9	0.02132	**125.6**	1.6	0.00019
	0.001%	**91.8**	0.9	0.00972	**176.7**	4.6	0.00002
	0.003%	**91.6**	3.0	0.02544	**184.5**	12.9	0.00039
DNA	**0%**	**100**	5.2	-	**100**	5.2	-
	0.0003%	**107.2**	12.6	0.40816	**106.3**	3.0	0.14577
	0.001%	**97.6**	9.3	0.71530	**131.9**	2.5	0.00068
	0.003%	**101.0**	13.9	0.91584	**135.7**	8.2	0.00316

Statistics: student t test referring to the control: $p < 0.05$ = significant effect;

$p < 0.01$ = very significant effect

DMSO was tested simultaneously at 0.03%, 0.1 and 0.3%, and did not significantly affect the levels of cellular proteins, ATP or DNA in cultured fibroblasts (data not shown).

10. In summary, Table 2 clearly demonstrates that the supercritical CO_2 extract of Schisandra chinensis at 0.0003%, 0.001% and 0.003% significantly enhances the levels of cellular proteins, ATP and DNA in cultured human fibroblasts, in contrast to the aqueous extract.

These results demonstrate that the supercritical CO_2 extract of Schisandra chinensis has dose-dependent revitalizing activity on human fibroblasts cultured *in vitro*,

whereas the aqueous extract shows no statistically significant effect.

11. Further, on the basis of the above *in vitro* test results, combined with those already disclosed in the Examples of the present specification, one skilled in the art would understand that the unexpected properties of the supercritical solvent extract of Schisandra chinensis would uniquely provide the claimed anti-ageing effects on human skin.

12. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Marie-France Zambaux

Date